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Improving Hashimoto's thyroiditis by eradicating *Blastocystis hominis*: Relation to IL-17

Hanaa Tarek El-Zawawy, Huda Fahmy Farag, Mona Mohamed Tolba and Hanaa Abdalbasit Abdalsamea

Abstract

Background: Hashimoto's thyroiditis (HT) is a common autoimmune disorder that causes significant morbidity. Interleukin (IL)-17 was identified as a major contributing factor in the pathogenesis of HT. *Blastocystis hominis* (BH) is a very common infection and has been shown to be associated with several diseases. Our aim was to determine serum IL-17 level in HT patients with and without BH infection and the effect of eradicating BH in patients with HT.

Methods: A prospective cohort study was conducted on 20 HT patients not infected with BH (group I), 20 HT patients infected with BH (group II), and 20 healthy patients (group III). Serum free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), thyroid peroxidase antibodies (anti-TPO), and IL-17 were performed by ELISA method and were repeated in group II after 6 weeks of eradication of BH.

Results: Patients with HT showed a significantly higher serum IL-17 compared with controls. IL-17 was significantly higher in HT patients infected with BH compared with HT patients not BH infected (mean 6.93 ± 2.83 pg/ml *versus* 3.25 ± 1.55 pg/ml, $p = 0.003$). After BH eradication TSH, anti-TPO, and IL-17 were significantly decreased (mean 14.76 ± 11.11 μIU/ml *versus* 9.39 ± 7.11 μIU/ml, $p < 0.001$; mean 308 ± 175.6 IU/ml *versus* 295.4 ± 167.1 IU/ml, $p = 0.006$; and mean 6.93 ± 2.83 pg/ml *versus* 6.45 ± 2.48 pg/ml, $p < 0.001$), respectively. Multivariate analysis after treating BH infection showed that IL-17 was significantly negatively correlated with FT3 (adjusted $p = 0.002$) and significantly positively correlated with anti-TPO (adjusted $p = 0.045$).

Conclusion: Treatment of BH infection ameliorates HT through reduction in IL-17, anti-TPO, and TSH.

Clinical trial registration number: PACTR201909495111649

Keywords: *Blastocystis hominis*, Hashimoto's thyroiditis, IL-17

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Correspondence to:
Hanaa Tarek El-Zawawy
Internal Medicine Department, Endocrinology Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt
hanaaelzawawy@yahoo.com

Huda Fahmy Farag
Mona Mohamed Tolba
Hanaa Abdalbasit Abdalsamea
Applied and Molecular Parasitology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt

Introduction

Hashimoto's thyroiditis (HT) is considered an important part of the spectrum of chronic autoimmune thyroid diseases mediated by the interaction between genetic and environmental factors.[1] It is considered the most common cause of hypothyroidism in areas with iodine sufficiency such as in Egypt.[2] HT is associated with thyroid autoantibodies production, the most common are thyroid peroxidase antibodies (anti-TPO) and thyroglobulin antibodies with various degrees of thyroid dysfunction.[3]

Formerly, HT was considered a T helper (Th) 1-mediated disease. After the identification of new Th cell subsets, including Th17 cells, HT was found to be mediated by Th17. Th17 cells secrete specific types of cytokines through which they exert their action. They are a major source of interleukin (IL)-17 and their effector functions are mediated mainly by this cytokine.[4]

Blastocystis hominis (BH) is the most common intestinal protozoan isolated in humans. Although this parasite remains asymptomatic in most of the

cases, yet it can function as an opportunistic pathogen and cause gastrointestinal disorders in immunocompromised patients. BH infection is associated with various diseases and it has been demonstrated that BH has the ability to modulate immune response.[5]

Th17 cells and their hallmark cytokine IL-17 were identified as major contributing factors in the pathogenesis of HT. Only a few studies addressed the relation between IL-17 and HT. It was interesting to study the level of IL-17 in HT patients with and without BH infection and observe the effect of eradication of BH on IL-17 level and the course of HT.

Aim

The aim of this work was to determine:

(1) The level of serum IL-17 in patients with HT with and without BH infection.
(2) The effect of treatment of BH infection on the level of serum IL-17 and thyroid parameters in patients with HT.

Patients and methods

This prospective cohort study was carried out on 40 patients who attended the endocrinology outpatient clinic in Alexandria Main University Hospital and 20 healthy individuals. They were categorized into 3 groups:

Group I: 20 Patients newly diagnosed with HT not infected with BH.
Group II: 20 Patients newly diagnosed with HT infected by BH. (BH infection was treated in this group).
Group III: 20 Healthy individuals without HT and negative for BH infection, age and sex matched, as a control group.

The diagnosis of HT was based on the presence of anti-TPO antibodies. We included both hypothyroid and euthyroid HT patients.

Criteria for exclusion included

(1) Parasitic infections (other than BH).
(2) Autoimmune diseases (Other than HT).
(3) Diabetes mellitus.
(4) Cardiovascular diseases.
(5) Malignancy and chronic inflammatory diseases.
(6) Pregnant or breastfeeding women.
(7) Patients receiving medications (including drugs affecting thyroid function).

Ethical issues

Approval was obtained from Ethical committees of both Faculty of Medicine (approval number: 0304272) and Medical Research Institute, Alexandria University. An informed written consent was obtained from each patient enrolled in the study.

Methods

All patients and controls were subjected to:

(1) History taking with special emphasis on gastrointestinal symptoms, symptoms of thyroid illness, and skin lesions if present.
(2) Clinical examination.
(3) Laboratory investigations:
  (a) Specific laboratory investigation for HT:
    (i) Free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), and Anti TPO performed by ELISA method.
    (ii) Serum IL-17 performed by ELISA method.
  (b) Stool samples for parasitological examination of BH and other parasitic infections using:
    (i) Wet mount technique; BH is stained with iodine and appears as a large vacuole in the middle of many small nuclei.
    (ii) Kato–Katz technique for exclusion of other parasitic infections.
    (iii) Sedimentation concentration technique followed by examination of the precipitate by saline wet mount and Lugol's iodine wet mount.
  (c) Routine investigations (Complete blood count (CBC), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), albumin, bilirubin, cholesterol, Triglycerides (TG), Blood urea nitrogen (BUN), creatinine).

In group II, BH infection was treated using 500 mg nanazoxid 3 tablets/day for 3 days then clinical examination and laboratory investigations were repeated after 6 weeks of eradication of BH infection.

Statistical analysis

Sample size was calculated using G Power 3.1. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Significance of the obtained results was judged at the 5% level. The chi-squared test for categorical variables was used to compare between different groups. The Monte Carlo correction for chi-squared was used when more than 20% of the cells had an expected count less than 5. The *F* test for normally distributed quantitative variables was used to compare between more than two groups and the *post hoc* test (Tukey) for pairwise comparisons. A paired *t*-test for normally distributed quantitative variables was used to compare between the means of two groups. The Pearson coefficient was used to correlate between two normally distributed quantitative variables. A p value < 0.05 was considered statistically significant. The Kruskal–Wallis test for abnormally distributed quantitative variables was used to compare between more than two studied groups, and *post hoc* (Dunn's multiple comparisons test) for pairwise comparisons. The Wilcoxon signed ranks test for abnormally distributed quantitative variables was used to compare between two periods. Regression analysis was used to detect the most independent/affecting factor for IL-17. Data were fed into the computer and analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp).

Results

The study included 40 patients with HT and 20 healthy patients, their ages ranged from 19 to 57 years. They were divided into three groups, group I included 20 patients with HT not infected with BH (19 females and 1 male) with a mean age of 37.70 ± 8.41 years, group II included 20 patients with HT infected with BH (19 females and 1 male) with mean age 36.0 ± 11.66 years, and group III included 20 healthy individuals without HT and negative for BH infection with matched age and sex.

All patients in groups I and II had a history of fatigue, nine patients in group I and seven patients in group II had a history of constipation, while six patients in group II had a history of diarrhea. No history of skin lesions among patients. Control patients in group III showed no significant history.

Patients in both groups I and II had no goiter. In group II, both systolic and diastolic blood pressures were significantly higher than in the control group (mean 135.5 ± 19.75 mmHg *versus* 121.9 ± 6.35 mmHg, $p = 0.011$) and (mean 84.10 ± 11.47 mmHg *versus* 82.80 ± 6.38 mmHg, $p = 0.011$), respectively.

Thyroid function results in the 3 studied groups as shown in Table 1, revealed that in comparison with the control group, mean TSH level was significantly higher in group I (17.51 ± 23.11 µIU/ml *versus* $2.63 \pm 0.7.8$ µIU/ml, $p = 0.001$), and in group II (14.76 ± 11.11 µIU/ml *versus* 2.63 ± 0.78 µIU/ml, $p = 0.001$). Mean FT4 was significantly lower in group I in comparison with group III (1.04 ± 0.48 ng/dl *versus* 1.35 ± 0.27 ng/dl, $p = 0.009$), in group II mean FT4 was 1.19 ± 0.38 ng/dl and showed no significant difference to the control group. Mean FT3 was significantly lower in group II in comparison with group III (1.98 ± 0.92 pg/ml *versus* 3.01 ± 0.69 pg/ml, $p = 0.001$), in group I mean FT3 was 2.54 ± 0.97 pg/ml and showed no significant difference to the control group.

Results of anti-TPO antibodies showed that it was significantly higher in groups I and II than in the control group (mean 436.6 ± 286.6 IU/ml *versus* 17.65 ± 5.66 IU/ml and mean 308.0 ± 175.6 IU/ml *versus* 17.65 ± 5.66 IU/ml, respectively). There was no significant difference in anti-TPO antibodies between groups I and II.

As shown in Figure 1, all patients with HT exhibited higher levels of serum IL-17 compared with normal patients. In comparison with the control group, serum IL-17 was significantly higher in patients with HT without BH infection (Group I) (mean 3.25 ± 1.55 pg/ml *versus* 1.41 ± 0.88 pg/ml, $p = 0.002$), as well as in patients with HT infected with BH (Group II) (mean 6.93 ± 2.83 pg/ml *versus* 1.41 ± 0.88 pg/ml, $p < 0.001$). Moreover, patients with HT who were infected with BH (Group II) showed a significant higher level of serum IL-17 compared with those with HT without BH infection (Group I; mean 6.93 ± 2.83 pg/ml *versus* 3.25 ± 1.55 pg/ml, $p = 0.003$).

Pearson's correlation in total sample size revealed that, serum IL-17 showed a significant positive correlation with anti-TPO ($p = 0.01$), whereas it showed a significant negative correlation with FT3 ($p = 0.003$).

Table 1. Comparison between the three studied groups regarding thyroid function.

	Group I ($n=20$)	Group II ($n=20$)	Group III ($n=20$)	Test of significance	p
TSH (μIU/ml)					
Min.–Max.	0.18–91.0	2.14–49.0	1.19–4.20	H = 29.694*	<0.001*
Mean ± SD.	17.51 ± 23.11	14.76 ± 11.11	2.63 ± 0.78		
Median	8.12	12.15	2.55		
Sig. bet. grps	$p_1=0.262$, $p_2<0.001$*, $p_3<0.001$*				
FT3 (pg/ml)					
Min.–Max.	0.41–4.70	0.60–3.17	1.80–4.40	F = 6.997*	0.002*
Mean ± SD.	2.54 ± 0.97	1.98 ± 0.92	3.01 ± 0.69		
Median	2.63	2.10	2.90		
Sig. bet. grps	$p_1=0.109$, $p_2=0.221$, $p_3=0.001$*				
FT4 (ng/dl)					
Min.–Max.	0.10–2.30	0.70–1.90	0.98–1.90	H = 7.197*	0.027*
Mean ± SD.	1.04 ± 0.48	1.19 ± 0.38	1.35 ± 0.27		
Median	1.10	1.20	1.40		
Sig. bet. grps	$p_1=0.438$, $p_2=0.009$*, $p_3=0.066$				

F, F for ANOVA test, pairwise comparison between each two groups was performed using *post hoc* test (Tukey); FT3, free triiodothyronine; FT4, free thyroxine; H, H for Kruskal Wallis test, pairwise comparison between each two groups was performed using *post hoc* test (Dunn's for multiple comparisons test); *p*, *p* value for comparing between the three studied groups; p_1, *p* value for comparing between the Group I and Group II; p_2, *p* value for comparing between the Group I and Group III; p_3, *p* value for comparing between the Group II and Group III; SD, standard deviation; Sig. bet. grps, significance between groups; TSH, thyroid stimulating hormone.
*Statistically significant at $p \leq 0.05$.

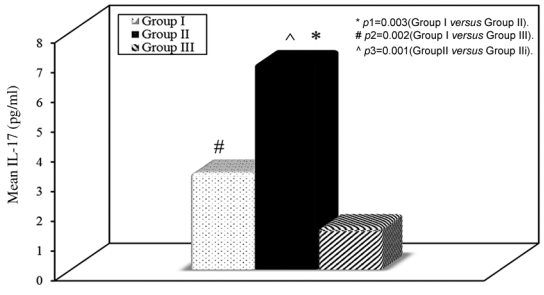

Figure 1. Comparison between the three studied groups according to interleukin (IL)-17.

Multiple regression analysis, shown in Table 2, for parameters affecting serum IL-17 in patients with HT infected with BH (before treatment) showed that FT3 was significantly negatively correlated with IL-17 (adjusted $p=0.011$) and anti-TPO was significantly positively correlated with IL-17 (adjusted $p=0.037$).

After treatment of Blastocystis hominis *infection*

After treatment of the BH infection, 10 patients in group II reported an improvement in fatigue, while constipation has improved in five patients only, and diarrhea disappeared in all six patients.

In group II, both systolic and diastolic blood pressures remain significantly higher than in the control group (mean 135.1 ± 18.62 mmHg *versus* 121.9 ± 6.35 mmHg, $p=0.010$) and (mean 86.75 ± 11.67 mmHg *versus* 82.80 ± 6.38 mmHg, $p=0.010$), respectively. There was no significant

Table 2. Univariate and multivariate analyses for the parameters affecting serum IL-7 in total sample (before BH infection treatment).

IL-7	Univariate		#Multivariate	
	Beta (95% CI)	***p***	**Beta (95% CI)**	***p***
Anti-TPO	0.004* (0.001–0.007)	0.010*	0.003* (0.0–0.006)	0.037*
FT3	–1.181* (–1.94 to –0.417)	0.003*	–1.003* (–1.7 to –0.243)	0.011*

Beta, standardized coefficients; BH, *Blastocystis hominis*; CI, confidence interval; FT3, free triiodothyronine; IL-7, interleukin-7.
#All variables with $p < 0.05$ was included in the multivariate.
*Statistically significant at $p \leq 0.05$.

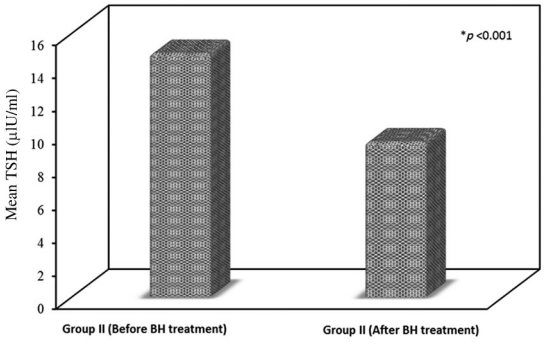

Figure 2. Comparison between thyroid stimulating hormone (TSH) levels in group II before and after treatment of *Blastocystis hominis* (BH) infection.

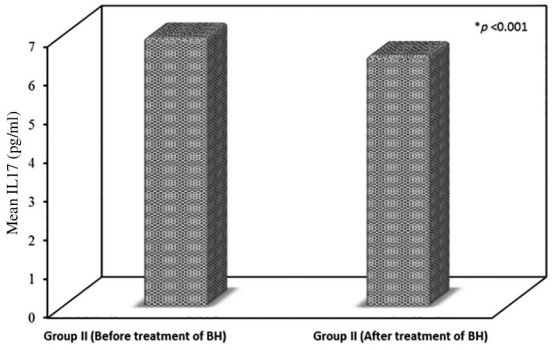

Figure 3. Comparison between serum interleukin (IL)-17 in patients with Hashimoto's thyroiditis infected with *Blastocystis hominis* (BH) (Group II) before and after treatment of BH infection.

difference in both systolic and diastolic blood pressures in group II before and after treatment of BH infection.

TSH was significantly decreased after treatment of the BH infection (mean 14.76 ± 11.11 µIU/ml *versus* 9.39 ± 7.11 µIU/ml, $p < 0.001$) as shown in Figure 2, with no significant difference in FT3 and FT4.

In group II, anti-TPO was decreased significantly after treatment of the BH infection with a mean of 295.4 ± 167.1 IU/ml *versus* 308.0 ± 175.6 IU/ml ($p = 0.006$).

Also in group II, as shown in Figure 3, after treatment of the BH infection in patients with HT, serum IL-17 was significantly decreased in comparison with its level before treating the BH infection (mean 6.45 ± 2.48 pg/ml *versus* 6.93 ± 2.83 pg/ml, $p < 0.001$).

Pearson's correlation in total sample size after eradication of BH revealed that serum IL-17 showed a significant positive correlation with anti-TPO ($p = 0.013$; Figure 4), whereas serum IL-17 showed a significant negative correlation with FT3 ($p = 0.001$; Figure 5).

Thus, it was shown in group II that treatment of a BH infection in patients with HT has resulted in a significant reduction in serum TSH, anti-TPO, and IL-17.

Multiple regression analysis, shown in Table 3, for parameters affecting serum IL-17 in patients with HT after treatment of BH infection revealed that FT3 was significantly negatively correlated with IL-17 (adjusted $p = 0.002$) and anti-TPO was significantly positively correlated with IL-17 (adjusted $p = 0.045$).

Multivariate analysis results showed that serum anti-TPO and FT3 were significant independent variables affecting serum level of IL-17 in patients with HT.

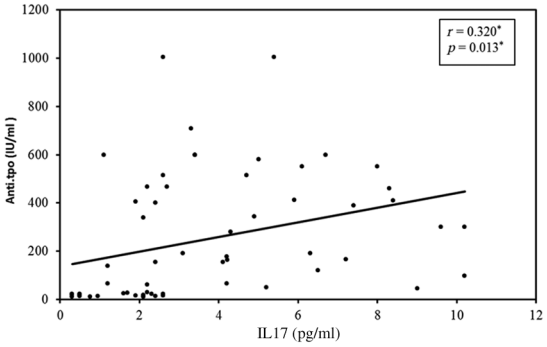

Figure 4. Correlation between interleukin (IL)-17 and thyroid peroxidase antibodies (anti-TPO) in group II after BH infection treatment.

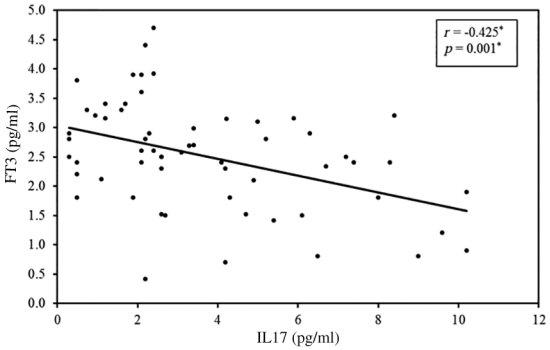

Figure 5. Correlation between interleukin (IL)-17 and free triiodothyronine (FT3) in group II after BH infection treatment.

Discussion

IL-17 has been found to be altered in HT. IL-17 is one of the major effector cytokines implicated in the generation, survival, and expansion of Th17 cells.[6] The current study showed that serum IL-17 was significantly elevated in patients with HT. In agreement with our results, Li and colleagues[7] demonstrated a significantly higher concentration of IL-17 in patients with HT, similarly Esfahanian and colleagues[4] showed a significant increase in serum IL-17 levels in HT patients, suggesting a potential role of this cytokine in disease pathogenesis.

BH has a wide geographic distribution with a prevalence varying from 1.6% to 16.0% in developed countries and can reach 60% in developing ones. The incidence of BH infection in rural areas is approximately 7%, while the incidence in urban areas is about 3.4%.[5] In Egypt, a high prevalence of BH has been reported among asymptomatic patients (22.4%), as well as patients with diarrhea and immunosuppressed children (12.1%).[8] Factors affecting BH infection include poor hygienic and sanitary conditions and the consumption of contaminated food or water.[5]

The Center for Disease Control and Prevention recommends concentrated stool samples stained with wet mount or a trichrome stain for diagnosis of BH, and at least three separate samples should be taken before a negative result is confirmed.[9]

BH is a common enteric parasite and an emerging pathogen found in humans.[10] It has been shown that BH can modulate immune response and BH infection is associated with activation of immune cells and an increase in various types of interleukins.[11] Specifically, IL-17 is increased with BH infection. Wu and colleagues[12] demonstrated that IL-17 was highly expressed in the intestinal mucosa of mice infected with BH. This was in accordance with our results, which showed that serum IL-17 was significantly higher in patients with HT infected with BH more than those with HT without BH infection. Thus, IL-17 is suggested to be one of the proinflammatory cytokines stimulated by BH infection and can play a role in the immunomodulatory effects induced by BH. Hence, BH can stimulate autoimmunity and can trigger HT through increasing proinflammatory cytokines linked to autoimmune diseases, including HT.[11] Also, BH infection can increase intestinal permeability and cause a leaky gut, which is the etiopathogenetic factor in many, if not all, autoimmune conditions.[13]

In 2015, for the first time in the literature a case report was published showing that treatment of BH infection can prevent the development and further stop progression of HT.[14] Our results supported those of the case report, we showed that treatment of BH infection in patients with HT resulted in a significant reduction in TSH and anti-TPO, which subsequently improved HT. Also, serum level of IL-17, a cytokine thought to have a role in HT pathogenesis, was decreased significantly after BH eradication that further supported the improvement of HT following treatment of BH infection.

In the current study, univariate and multivariate analyses for the parameters affecting serum IL-17 in patients with HT before and after treatment of

Table 3. Univariate and multivariate analyses for the parameters affecting serum IL-7 in total sample (after BH infection treatment).

IL-7 (after BH treatment)	Univariate		#Multivariate	
	Beta (95% CI)	*p*	Beta (95% CI)	*p*
Anti-TPO	0.003* (0.001–0.006)	0.013*	0.003* (0.0–0.005)	0.045*
FT3	−1.26* (−1.9 to −0.554)	0.001*	−1.113* (−1.81 to −0.41)	0.002*

Anti-TPO, thyroid peroxidase antibodies; Beta, standardized coefficients; CI, confidence interval; FT3, free triiodothyronine; IL-7, interleukin-7.
#All variables with $p < 0.05$ was included in the multivariate.
*Statistically significant at $p \leqslant 0.05$.

BH infection showed that serum IL-17 was significantly negatively correlated with FT3. Altamemi and colleagues[15] showed a significant positive correlation between IL-17 and TSH level, while they failed to demonstrate any significant correlation between IL-17 and both FT3 and FT4. On the other hand, Konca Degertekin and colleagues[6] showed a significant negative correlation between IL-17 and TSH, which they explained by the fact that hypothyroidism is known to be associated with depressed humoral and cell-mediated immunity and that hypothyroid HT patients in their study had a depressive effect on IL-17 with lower levels of IL-17 compared with euthyroid HT patients.[16] Our finding of the significant inverse correlation between IL-17 and FT3 can be justified by the possible suppressive effect of IL-17 on the deiodinase enzyme, which catalyzes the peripheral conversion of T4 to T3. This suppressive effect of IL-17 on the deiodinase enzyme is based on the fact that demonstrates cytokine induced alteration of the expression and activity of deiodinases.[17]

In addition, in the present study multiple regression analysis showed that serum IL-17 was significantly positively correlated with anti-TPO. Similarly, Bossowsk and colleagues[18] showed a significant positive correlation between IL-17 and anti-TPO. This can be explained by the suggested role of IL-17 in the immunopathogenesis of HT. However, this was in contrast to the results of Gerenova and colleagues[19] who did not find a significant correlation between serum IL-17 and anti-TPO; this might be because they included in their study HT patients with negative anti-TPO in whom fine needle aspiration biopsy was performed and HT was diagnosed based on the typical cytological features of autoimmune thyroiditis.

Thus, in our study, multivariate analysis of the results showed that serum anti-TPO and FT3 were significant independent variables affecting serum level of IL-17 in patients with HT.

Our study has two limitations; the first limitation is the small sample size that can probably lead to a loss of statistical significance at certain points, the second limitation is not assessing the changes in thyroid gland by ultrasonography before and after treatment of BH infection. However, our current study is a pilot observational study that acts as a base for further recommended larger studies addressing the relationship between HT and BH infection.

The major strength of the current study is that it is the first study to suggest that BH eradication can improve HT.

Conclusion

High serum IL-17 level in HT patients infected with BH supports the potential role of BH in the development of HT. Furthermore, BH eradication in patients with HT resulted in a reduction of serum IL-17 and improved thyroid parameters. Hence, treating BH infection can ameliorate HT and even stop its progression. Yet, such a relation is not definitively proven, and our findings can provide the basis for further multifaceted prospective studies with larger sample sizes.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

ORCID iD

Hanaa Tarek El-Zawawy https://orcid.org/0000-0002-2778-7689

Supplemental material

Supplemental material for this article is available online.

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